REMARKS

The Office Action mailed 13 February 2009, has been received and its contents carefully noted. Claims 1-19 and 21-22 were pending and claims 1-19 and 21-22 were rejected. By this amendment, claims 1, 2, 4-9 and 21 remain in this application. Claim 20 is withdrawn as being drawn to a non-elected invention. Also, claims 3, 10-19 and 22 have been canceled without prejudice or disclaimer. Applicant reserves the right to file a divisional application for any of the deleted and/or cancelled claims. By this Response, the claims have been amended as follows:

- Claims 1, 2, 20 and 21 have been amended to correct typographical and grammatical errors noted by the Examiner. Specifically, the terms "hybridising", "hybridised", "hybridisation", and "analysing" have been amended to read --hybridizing--, --hybridized--, --hybridization--, and --analyzing--, respectively. In addition, claims 1, 6, 20 and 21 have been amended to change "labelling" and "labelled" to --labeling—and--labeled--, respectively.
- Claim 10 has been canceled without prejudice or disclaimer.
- Claim 1 has also been amended to clarify that the methylation-sensitive restriction endonucleases cut unmethylated sequences but not methylated sequences and that the methylation-specific endonucleases cut methylated sequences but not unmethylated sequences. The amendment is fully supported by the description and Figures as originally filed.
- In claim 2, step (iii) has been amended to indicate comparing the one or more ratios determined in step (ii) rather than step (j) as previously recited.
- Claim 4 has been amended to replace "CpG specific endonuclease" with --methylation specific endonucleases-- to provide proper antecedent basis with claim 1, upon which claim 4 depends. The amendment is fully supported by the description, claims and Figures, for example, Figure 1 as originally filed.
- Claim 5 has been amended for clarity. Specifically, the term "cocktail" has been deleted.
- Claim 7 has been amended to delete reference to the phrase "a disease such as".
- Claim 8 has been amended to indicate that the first label, second label or both are chemically reactive fluorophores. The amendment is made to provide proper antecedent basis with respect to claim 1, upon which claim 8 depends.
- Claim 9 has been amended to indicate that the chemically reactive fluorophores are independently Cy 3 or Cy 5.
- Claim 21 has been amended to delete the ambiguous phrases such as "for example a disease such as but not limited to cancer, diabetes, Alzheimer's disease, schizophrenia or the like" and to delete reference to Phi 29 DNA polymerase. Further, the claim has been amended to indicate that the phenotype is a disease phenotype.

Support may be found in the specification and the claims as originally filed. No statutory new matter has been added. Therefore, reconsideration and entry of the claims as amended are respectfully requested.

Objection to the Specification

The Examiner objected to the Specification for containing embedded hyperlinks.

Applicants respectfully submit that the objection to the Specification may be withdrawn in view of the amendment to the Specification.

Claim Objections

The Examiner has objected to claims 1-19, 21 and 22 for various informalities.

Applicants respectfully submit that the claim objections may be withdrawn in view of the amendments to the claims.

Claim Rejections Under 35 U.S.C. 112, second paragraph

The Examiner rejected claims 2-4, 7-9, 17-19 and 21 under 35 U.S.C. 112, second paragraph, as being indefinite. With respect to each rejection, Applicants respectfully submit the following:

(a) The Examiner alleged that there is insufficient antecedent basis for "step j" in step iii) of claim 2.

In response, Applicants have amended "step j" to read "step ii)" to provide for proper antecedent basis for this limitation in the claim.

(b) The Examiner alleged that there is insufficient antecedent basis for "step 1)" in line 1 of claim 3.

In response, Applicants have canceled claim 3 without prejudice or disclaimer.

(c) The Examiner alleged that there is insufficient antecedent basis for "the CpG specific

endonuclease" in line 1 of claim 4.

In response, Applicants have amended claim 4 to replace "CpG specific endonuclease" with "methylation specific endonuclease" to provide sufficient antecedent basis for the limitation in this claim.

(d) The Examiner alleged that the phrases "such as" and "for example" in claims 7, 17, and 21, render the claims indefinite because it is unclear whether the limitation(s) following the phrases are part of the claimed invention.

In response, Applicants have deleted the ambiguous phrases noted by the Examiner in claims 7 and 21. Claim 17 has been canceled without prejudice or disclaimer.

(e) The Examiner alleged there is insufficient antecedent basis for "said probe" in line 1 of claims 8 and 18.

In response, Applicants have amended the term "said probe" to read "the first label, second label or both are" and claim 18 has been canceled without prejudice or disclaimer.

(f) The Examiner alleged that there is insufficient antecedent basis for "said fluorophore" in lines 1-2 of claims 9 and 19.

In response, Applicants have amended the term "said fluorophore" to read "said chemically reactive fluorophores" and claim 19 has been canceled without prejudice or disclaimer.

In view of the above, Applicants respectfully submit that the claims, as amended, are clear and definite and the rejections under 35 U.S.C. 112, second paragraph, should properly be withdrawn.

Moot Rejections

The Examiner rejected claims 10, 13, 14 and 16-19 under 35 U.S.C. 102(b) as being anticipated by Yan (J Nutr. 2002 Aug; 132 (8 Suppl): 2430S-2434S). The Examiner also

rejected claims 10, 13, 14 and 16-19 under 35 U.S.C. 102(e) as being anticipated by Huang (US 6,605,432). The Examiner rejected claim 15 under 35 U.S.C. 103(a) as being unpatentable over Yan or Huang in view of Sutcliffe (US 6,110,680).

Applicants have canceled claims 10-19. Therefore, these rejections should be withdrawn as being moot.

The Claimed Invention – Provides Unmethylated Fraction for Analysis

The present invention, as claimed, is concerned with examination of the unmethylated fraction of genomic DNA. Applicants have amended clam 1 to clarify that the methylation-*sensitive* restriction endonucleases cut *unmethylated* sequences but not methylated sequences and the methylation-*specific* endonucleases cut *methylated* sequences but not unmethylated sequences. Thus, the claims, as amended, essentially encompass the subject matter as defined in the middle portion of Figure 1a of the instant specification under "Unmethylated Fraction". Specifically, according to the present invention and as schematically shown in the middle portion of Figure 1a, the unmethylated sequences are cut (step b of claim 1) and then the methylated sequences of the ligated sequences are cut (step d of claim 1). Cleavage with the methylation-*sensitive* restriction endonucleases first and then cleavage with the methylation-*specific* endonucleases results in the unmethylated fraction for analysis.

Interrogation of a hypermethylated fraction of DNA is completely different from interrogation of the unmethylated fraction. Information on the unmethylated fraction of DNA can not be envisaged from information obtained from the hypermethylated fraction, and vice versa. The cited documents do not teach or suggest interrogation of the unmethylated fraction of DNA in accordance with the present invention.

It should be noted that restriction enzymes such as HpaII, Hin6l, etc., are methylation *sensitive* as they cut only unmethylated DNA and restriction enzymes such as McrBC are methylation *specific* enzymes as they cut only methylated DNA.

The Cited Documents – Provides Hypermethylated Fraction for Analysis

As shown in the right panel of Figure 1a under "Hypermethlyated Fraction", cleavage

with the methylation-*specific* endonucleases first and then cleavage with the methylation-*sensitive* restriction endonucleases results in the methylated fraction for analysis.

Yan (J Nutr. 2002 Aug; 132 (8 Suppl): 2430S-2434S)

The Examiner characterizes Yan as teaching methods of analyzing the methylation states of nucleotide sequences. Applicants respectfully submit that this oversimplification by the Examiner mischaracterizes the disclosure of Yan. In particular, Yan focuses on hypermethylated DNA sequences in cancer and teaches methods of analyzing the hypermethylation state of nucleotide sequences. This is clearly evident from the full disclosure of Yan, in particular, see the last line of the abstract and Figure 2.

In fact, Yan teaches away from amplifying and analyzing unmethylated CpG sites as Yan discloses:

> The basis of the DMH assay builds on the use of methylation-sensitive endonucleases to discriminate between the methylation profile of a tumor sample from its paired normal control. For this scheme to work, we need to ascertain the presence of restriction enzyme recognition sites in the CGI clones. We randomly selected 50 CGI clones from those that are arrayed on the CGI microarray. Over 90% of these clones contain at least one of the three restriction sites. This information prompted us to reevaluate our original DMH strategy (19) whereby a subtractive hybridization step, which is both costly and laborious to perform, is implemented to reduce the presence of repetitive sequences in the prepared amplicons. We reasoned that the majority of the CpG sites present in these repetitive fragments would be unmethylated in the normal genome and possibly hypomethylated in the tumor genome. Therefore, being able to remove by enzymatic restriction close to 90% of the repetitive sequences would eliminate the necessity of having a subtractive hybridization step. We compared the hybridization intensities of both high- and low-copy number CGI loci prepared with and without this step and found them to be quite similar. As such, our revised DMH assay has two methylation-sensitive restriction steps (to increase coverage and to safeguard against possible incomplete digestion) in place of the subtractive hybridization step, followed by one methylation-sensitive restriction step. An added advantage of this modification is that it removes a tedious step from the DMH assay and makes the whole protocol amenable to a high-throughput sample preparation scheme.

See p. 2432S, 1st full para, left col. (emphasis added).

In short, Yan teaches a DMH assay which removes hypomethylated/unmethylated sequences by enzymatic restriction so that a costly subtractive hybridizing step need not be

implemented. Clearly, the principle of operation of Yan is to perform one or more methylation-sensitive restriction steps followed by a methylation-sensitive restriction step for the intended purpose of analyzing only the hypermethylated profile of breast tumor and normal samples.

Therefore, Yan teaches away from the sequential use of a methylation-sensitive restriction endonuclease followed by one or more methylation-specific endonucleases to interrogate the unmethylated fraction of DNA.

Further Remarks for Clarification

The Examiner also alleges that Yan discloses digesting the genomic test nucleotide sequences and separately digesting the genomic control sequences with one or more frequent cutting restriction endonucleases such as Mse1, a TTAA cutter. It is important to note that MseI is not a methylation-sensitive restriction enzyme according to the present invention. A methylation-sensitive restriction endonuclease cuts specific unmethylated DNA sequences but leaves the same methylated sequences intact. Because its recognition site (TTAA) rarely occurs in GC-rich regions, most GC-rich CGI remain intact after this restriction. See Yan, page 2432s, left hand paragraph, lines 1-4. Thus, MseI it is a frequent cutting restriction enzyme that does not cut in CG-rich regions and is not a methylation-sensitive restriction enzyme. Consequently, Yan does not disclose or suggest digesting DNA with a methylation sensitive restriction endonuclease as recited in step b) of instant claim 1.

Huang (US 6,605,432)

The Examiner alleges that Huang teaches methods of analyzing the methylation states of nucleotide sequences. Similar to the Examiner's characterization of Yan, Applicants respectfully submit that the Examiner's gross oversimplification of Huang is a mischaracterization. In particular, Huang discloses a DMH assay which is essentially the same as that described in Yan.[1] Similar to Yan, the principle of operation of Huang is to perform one or more methylation-sensitive restriction steps followed by a methylation-sensitive restriction step for the intended purpose of analyzing only the hypermethylated profile of breast tumor and normal samples. See

[1] It is noted that the inventor Huang is the same as the last listed author of Yan.

e.g. claim 1, and the Examples.

Thus, Huang also teaches away from the sequential use of a methylation-sensitive restriction endonuclease followed by one or more methylation-specific endonucleases to interrogate the unmethylated fraction of DNA.

Claim Rejections Under 35 U.S.C. 103(a)

The Examiner rejected claims 1, 4-9 and 22 under 35 U.S.C. 103(a) as being unpatentable over Yan or Huang in view of Chotai (J Med Genet. 1998 Jun;35(6):472-5). Specifically, the Examiner correctly recognized that neither Yan nor Huang teach the successive digestion of a nucleic acid sample with a methylation-sensitive restriction enzyme followed by a methylation-specific restriction enzyme. Thus, the Examiner deemed that in view of the disclosure of Chotai it would have been obvious to do the reverse, i.e. use a methylation-sensitive restriction enzyme in the first digestion step and of Yan or Huang.

1. The disclosure of Yan or Huang can not be modified by the disclosure of Chotai

THE PROPOSED MODIFICATION CANNOT CHANGE THE PRINCIPLE OF OPERATION OF A REFERENCE

Applicants respectfully submit that a prima facie case of obviousness has not been established. Specifically, Applicants respectfully submit that using a methylation-sensitive restriction enzyme in the first digestion step and of Yan or Huang would change the principle of operation of the invention of Yan or Huang. In particular, as noted above, the principle of operation of the invention of Yan and Huang is to perform one or more methylation-sensitive restriction steps followed by a methylation-sensitive restriction step in order to obtain only the hypermethylated sequences for analysis. Using a methylation-sensitive restriction enzyme in the first digestion step would change the principle of operation as the unmethylated sequences would be obtained. If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims prima facie obvious. In re Ratti, 270 F.2d 810, 123 USPQ 349 (CCPA 1959); MPEP 2143.01.

Therefore, Applicants respectfully submit that a prima facie case of obviousness has not been established.

THE PROPOSED MODIFICATION CANNOT RENDER THE PRIOR ART UNSATISFACTORY FOR ITS INTENDED PURPOSE

Applicants respectfully submit that there is no suggestion or motivation to use a methylation-sensitive restriction enzyme in the first digestion step of Yan or Huang as the modification would render the invention of Yan or Huang unsatisfactory for its intended purpose. Specifically, the intended purpose of Yan and Huang is to analyze only the hypermethylated profile of breast tumor and normal samples. Using a methylation-sensitive restriction enzyme in the first digestion step would result in the unmethylated sequences rather than the hyper methylated sequences. Fractions of unmethylated sequences are unsatisfactory for analyzing hypermethylated sequences. Consequently, using a methylation-sensitive restriction enzyme in the first digestion step of Yan or Huang would result in unmethylated sequences which are unsatisfactory for analyzing the hypermethylated profile of breast tumor and normal samples. If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. In re Gordon, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984); MPEP 2143.01.

Thus, Applicants respectfully submit that the disclosures of Yan and Huang can not be modified such that a methylation-sensitive restriction enzyme is used in the first digestion step. Therefore, the claimed invention is unobvious.

EVEN IF COMBINED, THE COMBINATION DOES NOT RESULT IN THE CLAIMED INVENTION AS A WHOLE

The Examiner alleges that Chotai teaches the use of a methylation-sensitive restriction enzyme (NotI) in conjunction with a methylation-specific restriction enzyme (McrBC) to differentiate methylation status of target nucleic acids. Applicants respectfully submit that the Examiner does not appreciate the disclosure of Chotai. Specifically, Chotai does not teach or suggest the use of a methylation-sensitive restriction enzyme (NotI) in conjunction with a

methylation-specific restriction enzyme (McrBC). Instead, Chotai teaches the use of a methylation-sensitive restriction enzyme (NotI) **or** a methylation specific restriction enzyme (McrBC). This is clearly evident throughout the Chotai disclosure. See, for example:

> We have developed a novel and rapid diagnostic test for PWS and AS based on differential digestion of expressed (paternally imprinted) SNRPN sequences by the methylation-sensitive endonuclease NotI **or** repressed (maternally imprinted) SNRPN sequences by the methylation requiring nuclease McrBC, followed by PCR amplification of the SNRPN promoter.

see 1st sentence, second paragraph of Abstract (emphasis added);

> This strategy uses either NotI to digest unmethylated (paternally imprinted) DNA **or** McrBC to digest methylated (maternally imprinted) DNA followed by PCR amplification of the promoter and first exon of SNRPN to diagnose AS or PWS respectively.

See last sentence immediately before method section (emphasis added); and

> PCR amplification was performed on 100 ng DNA either undigested (amplification control) or digested with NotI **or** McrBC. PCR included both "diagnostic" primers S1 and S2 (to amplify a 1088).

See first full sentence in second paragraph following PCR based methylation analysis (emphasis added).

As is evident from these passages, Chotai teaches that the DNA of patients suspected of Prader-Willi syndrome (PWS) or Aangelman syndrome (AS) can be diagnosed by either the methylation-sensitive endonuclease NotI or by the methylation requiring nuclease McrBC to diagnose and differentiate between PWS and AS.

Chotai is concerned with the methylation status of a single locus. Chotai is not concerned with the collective interrogation of the entire unmethylated fraction of genomic DNA. Similarly, Chotai is not concerned with the collective interrogation of the methylated fraction of DNA. Chotai discloses treating DNA with either a methylation sensitive endonuclease or a methylation requiring endonuclease.

Chotai does not disclose or suggest sequential treatment with multiple restriction enzymes at all. Thus, Chotai does not teach or suggest use of a methylation-sensitive restriction enzyme in a first digestion step followed by use of a methylation-specific restriction enzyme in a

subsequent digestion step in a single assay for a given sample. Nowhere does Chotai teach or suggest which restriction enzyme should be used in a DMH assay according to Yan or Huang. Therefore, even if Yan or Huang was combined with Chotai, the combination would not result in the claimed invention as a whole.

Since the disclosures of Yan or Huang can not be combined with the disclosure of Chotai and even if combined, the combination would not result in the claimed invention as a whole, the claimed invention is unobvious and the rejection under 35 U.S.C. 103(a) should properly be withdrawn.

Dean (US 6,617,137)

The Examiner rejected claims 2 and 3 under 35 U.S.C. 103(a) as being unpatentable over Yan or Huang in view of Chotai and in further view of Dean et al. (US 6,617,137). The Examiner also rejected claims 11, 12, and 21 as being unpatentable over Yan or Huang in view of Dean.

Applicants respectfully submit that Dean fails to alleviate the deficiencies of Yan, Huang, and Chotai, alone or in combination. Specifically, nowhere does Dean teach or suggest an assay for analyzing unmethylated DNA wherein the unmethylated sequences are cut (step b of claim 1) and then the methylated sequences of the ligated sequences are cut (step d of claim 1). In fact, Dean does not even mention the methylation status of any DNA sequence anywhere in the entire document.

Since Dean fails to alleviate the deficiencies of Yan, Huang, and Chotai, the rejections of claims 2, 3, 11, 12 and 21 should properly be withdrawn.

Request for Interview

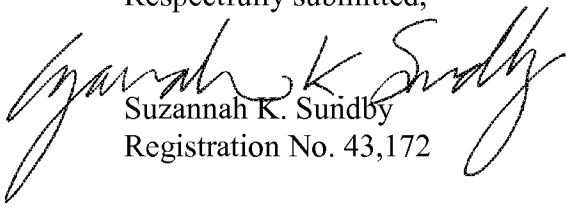
Either a telephonic or an in-person interview is respectfully requested should there be any remaining issues.

CONCLUSION

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Therefore, it is respectfully requested that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Official action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, in the event that additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. 1.136(a), and any fees required therefor are hereby authorized to be charged to **Deposit Account No. 024300**, Attorney Docket No. **034263.002**.

Respectfully submitted,



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